ARE ‘APPARENT’ SEX REVERSED CHINOOK SALMON A SYMPTOM OF GENOTOXICITY?

prepared by May, Bernie P

submitted to Science Program 2004

compiled 2005−01−06 12:42:57 PST

Project

This proposal is for the Science Program 2004 solicitation as prepared by May, Bernie P.

The submission deadline is 2005−01−06 17:00:00 PST (approximately 4.3 hours from now).

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Proposal Title	***ARE 'APPARENT' SEX REVERSED CHINOOK SALMON A SYMPTOM OF GENOTOXICITY?***	
Institutions	University of California, Davis NOAA Fisheries NW Fish Sci Center, Washington State University, Vancouver	*List each institution involved, one per line.*
Proposal Document		*You have already uploaded a proposal document. View it to verify that it appears as you expect. You may replace it by uploading another document*
Project Duration	***12 months***	

Is the start date a determining factor to the successful outcome of the proposed effort?
− No.
X Yes. Anticipated start date of this effort: ***2005−08−01***

Select all of the following study topics which apply to this proposal.
X life cycle models and population biology of key species
X environmental influences on key species and ecosystems
X relative stresses on key fish species
− direct and indirect effects of diversions on at−risk species
− processes controlling Delta water quality
− implications of future change on regional hydrology, water operations, and environmental processes
− water management models for prediction, optimization, and strategic assessments
X assessment and monitoring
X salmonid−related projects
− Delta smelt−related projects

Select as many keywords as necessary to describe this proposal (minimum of 3).
− ***adaptive management***
− ***aquatic plants***
− ***benthic invertebrates***
− ***biological indicators***
− ***birds***
− neotropical migratory birds
− shorebirds
− upland birds
− wading birds
− waterfowl
− ***climate***
− climate change
− precipitation
− sea level rise
− snowmelt
− ***contaminants / toxicants / pollutants***
X contaminants and toxicity of unknown origin
X emerging contaminants
− mercury

− nutrients and oxygen depleting substances
− organic carbon and disinfection byproduct precursors
− persistent organic contaminants
− pesticides
− salinity
− sediment and turbidity
− selenium
− trace metals
− ***database management***
− ***economics***
− ***engineering***
− civil
− environmental
− hydraulic
− ***environmental education***
− ***environmental impact analysis***
− ***environmental laws and regulations***
− ***environmental risk assessment***
X fish biology
− bass and other centarchids
− delta smelt
− longfin smelt
− other species
X salmon and steelhead
− splittail
− striped bass
− sturgeon
− ***fish management and facilities***
X hatcheries
− ladders and passage
− screens
− ***forestry***
X genetics
− ***geochemistry***
− ***geographic information systems (GIS)***
− ***geology***
− ***geomorphology***
− ***groundwater***
− ***habitat***
− benthos
− channels and sloughs
− flooded islands
− floodplains and bypasses
− oceanic
− reservoirs
− riparian
− rivers and streams
− shallow water
− upland habitat
− vernal pools
− water column
− wetlands, freshwater
− wetlands, seasonal
− wetlands, tidal
− ***human health***
− ***hydrodynamics***
− ***hydrology***
− ***insects***
− ***invasive species / non−native species / exotic species***
− ***land use management, planning, and zoning***
− ***limnology***
− ***mammals***
− large
− small
− ***microbiology / bacteriology***

− ***modeling***
− conceptual
− quantitative
− ***monitoring***
− ***natural resource management***
− ***performance measures***
− ***phytoplankton***
− ***plants***
− ***primary productivity***
− ***reptiles***
− ***restoration ecology***
− ***riparian ecology***
− ***sediment***
− ***soil science***
− ***statistics***
− ***subsidence***
− ***trophic dynamics and food webs***
− ***water operations***
− barriers
− diversions / pumps / intakes / exports
− gates
− levees
− reservoirs
− ***water quality management***
− ag runoff
− mine waste assessment and remediation
− remediation
− temperature
− urban runoff
− water quality assessment and monitoring
− ***water resource management***
− ***water supply***
− demand
− environmental water account
− water level
− water storage
− ***watershed management***
− ***weed science***
− ***wildlife***
− ecology
− management
− wildlife−friendly agriculture
− ***zooplankton***
− ***administrative***

Indicate whether your project area is local, regional, or system−wide. If it is local, provide a central ZIP Code. If it is regional, provide the central ZIP Code and choose the counties affected. If it is system−wide, describe the area using information such as water bodies, river miles, and road intersections.	
− local	ZIP Code:
− regional	ZIP Code: counties:
X system−wide	All California waterways in which Fall−Run Chinook salmon occur including the San Joaquin River and its' tributaries (Merced River, Tuolumne River, and Stanislaus River), the Sacramento River Basin and it's tributaries (American River, Feather River, Clear Creek, Battle Creek, and Yuba River).

Does your project fall on or adjacent to tribal lands?
No.

(Refer to California Indian reservations to locate tribal lands.)
If it does, list the tribal lands.

Has a proposal for this effort or a similar effort ever been submitted to CALFED for funding or to any other public agency for funding?
Yes.

If yes, complete the table below.

Status	Proposal Title	Funding Source	Amount	Comments
funded	*Sex−reversal in Central Valley Chinook salmon: occurrence and population genetic consequences*	*USFWS− Anadromous Fish Restoration Program*	*$212,936*	*The project has been successfully completed and has resulted in a peer−reviewed publication.*
pending	*Extension of Sex−reversal in Central Valley Chinook salmon: evaluation of potential introgression into threatened spring−run populations*	*USFWS− Anadromous Fish Restoration Program*		*Funding for this program is pending*

Has the lead scientist or principal investigator of this effort ever submitted a proposal to CALFED for funding or to any other public agency for funding?
Yes.

If yes, provide the name of the project, when it was submitted, and to which agency and funding mechanism if was submitted. Also describe the outcome and any other pertinent details describing the proposal's current status.

All applicants must identify all sources of funding other than the funds requested through this solicitation to support the effort outlined in their proposal. Applicants must include the status of these commitments (tentative, approved, received), the source, and any cost−sharing requirements. Successful proposals that demonstrate multiple sources of funding must have the commitment of the non−Science Program PSP related funding within 30 days of notification of approval of Science Program PSP funds. If an applicant fails to secure the non−Science Program PSP funds identified in the proposal, and as a result has insufficient funds to complete the project, CBDA retains the option to amend or terminate the award. The California Bay−Delta Authority reserves the right to audit grantees.

Status	Proposal Title	Funding Source	Period Of Commitment	Requirements And Comments

Are you specifically seeking non−federal cost−share funds for this proposal?
No.

In addition to the general funds available, are you targeting additional funds set aside specifically for collaborative proposals?
Yes.

List people you feel are qualified to act as scientific reviewers for this proposal and are not associated with CALFED.

Full Name	Organization	Telephone	E−Mail	Expertise
Charles C. Krueger	*Great Lakes Fishery Commission*	*(734) 662−3209 ext 12*	*ckrueger@glfc.org*	*fish biology, salmon and steelhead*
Bill Ardren	*US Fish and Wildlife Service*	*(360) 425−6072 ext 256*	*William_Ardren@r1.fws.gov*	*genetics*
Mark Bagley	*Environmental Protection Agency*	*(513) 569−7455*	*mark.bagley@epa.gov*	*contaminants / toxicants / pollutants, emerging contaminants*

Executive Summary

Provide a brief but complete summary description of the proposed project; its geographic location; project objective; approach to implement the proposal; hypotheses being tested; expected outcomes; and relationship to Science Program priorities. The Executive Summary should be a concise, informative, stand−alone description of the proposed project. *(This information will be made public on our website shortly after the closing date of this PSP.)*

Executive Summary Are ‘apparent sex reversed’ Chinook salmon a symptom of genotoxicity? Amount Requested: $143,735 Applicant: Dr. Bernie May, Adjunct Professor, Dept. of Animal Science, University of California, Davis. Tel.: (530) 754−8123 FAX: (530) 752−0175 Participants and Collaborators: B. May (UC Davis), K. Williamson (Northwest Fisheries Science Center− NOAA Fisheries), R. Phillips (Washington State University)

The primary goal of this research project is to facilitate implementation of the CALFED Ecosystem Restoration goal for fall−run Chinook salmon with respect to the relative importance of chemical stressors on population viability and genetic diversity. We will determine the chromosomal mechanism responsible for producing phenotypic female fall−run Chinook salmon in the Central Valley that test positive for Y−chromosome specific genetic markers. Using established fish−rearing and molecular genetic protocols developed during a previous study we will perform genetic and cytogenetic analyses of offspring produced from genetically normal and ‘apparent’ XY−female fall−run Chinook salmon. This research project involves three tasks. First, we will

harvest gametes and evaluate genotypic sex of phenotypic female and male fall−run Chinook salmon collected from the Merced River Fish Hatchery using two Y−chromosome specific markers, OtY1 and growth hormone pseudogene. By performing genetic screening of sex to determine which of the phenotypic females have a male genotype (XY−females) and which are genetically normal (XX) females, putative gamete donors will be selected for use in controlled breeding experiments. Second, we will evaluate the genetic sex of offspring produced from these crosses using the Y−chromosome markers, plus a recently published Y−chromosome marker, OtY2(WSU). Gross gonad morphology will be observed via necropsy to ascertain the phenotypic sex of offspring. In addition, whole blood will be collected from individuals to be used to create lymphocyte cultures for cytogenetic analyses. Third, the offspring produced between 'apparent' XY−female and normal male fish will be compared cytogenetically with the progeny of normal male and female fish using a previously established cytogenetic method. This will allow us to determine the chromosomal mechanism responsible for producing 'apparent' XY−female fall−run Chinook salmon. Evaluating the chromosomal changes incurred by XY−females should remove uncertainty regarding whether or not XY−female fish negatively impact population genetics and persistence and if they are a symptom of genotoxicity experienced by fall populations due to exposure to environmental contaminants. We will use a hypothesis driven approach to reduce uncertainty regarding the negative impact that 'apparent' sex−reversed individuals have on populations of this at−risk species. We will address the following hypotheses:

1. Is the 'apparent' sex−reversed phenotype observed in fall−run Chinook salmon due to a Y− to X−chromosome/autosome translocation? Ho: Male &female offspring of apparent XY−female fall−run Chinook salmon produce patterns of chromosome staining that are consistent with recombination of the male (Y−chromosome) specific markers to the X−chromosome/autosome.

2. Is the 'apparent sex−reversed' phenotype observed in fall−run Chinook salmon due to a Y−chromosome that has had the sex determining region deleted or inactivated? Ho: Male &female offspring of apparent XY−female fall−run Chinook salmon produce patterns of chromosome staining that are consistent with a Y−chromosome that lacks a functional sex−determining region.

3. Does the OtY2(WSU) Y−chromosome specific marker exhibit the same pattern of inheritance as two other male−specific markers, OtY1 and the growth hormone pseudogene, in a controlled mating between an 'apparent' XY−female and a genetically normal male? Ho: The Y−chromosome specific marker OtY2(WSU) is inherited in a manner consistent with Mendelian segregation. Specifically, does a controlled cross between a normal male (XY) and an 'apparent' XY−female produce a 1:3, female to male, genotypic sex ratio in the offspring?

The study of genetic mechanisms that alter sexual development of Central Valley Chinook salmon is within the CALFED Program goals of protecting and improving At−Risk Native Species populations and the CALFED Science Program goal of addressing uncertainties that influence management and developing performance measures. Our project will contribute information to the specific priority topic areas of life cycle models and population biology of, and relative stresses on target fish species. We hope to provide management agencies with information regarding the impact that 'apparent' sex−reversed fish have on reproduction, population genetics, and thus population persistence of fall−run Chinook. In addition to providing quarterly reports the information produced will be disseminated to the management and scientific communities through poster and oral presentations at local and national meetings, as well as publication in peer−reviewed journals.

Give additional comments, information, etc. here.

Applicant

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Instructions

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All information on this page is to be provided for the agency or institution to whom funds for this proposal would be awarded.

Applicant Institution	***University of California, Davis***	*This list comes from the project form.*
Applicant Institution Type	***public institution of higher education***	

Institution Contact

Please provide information for the primary person responsible for oversight of grant operation, management, and reporting requirements.

Salutation	***Dr.***
First Name	***Bernie***
Last Name	***May***
Street Address	***2237 Meyer Hall, Dept Animal Science, Univ of CA, One Shields Ave.***
City	***Davis***
State Or Province	***CA***
ZIP Code Or Mailing Code	***95616***
Telephone	***530−754−8123*** *Include area code.*
E−Mail	***bpmay@ucdavis.edu***

Additional information regarding prior applications submitted to CALFED by the applicant organization or agency and/or funds received from CALFED programs by applicant organization or agency may be required.

Personnel

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Instructions

Applicants must provide brief biographical sketches, titles, affiliations, and descriptions of roles, relevant to this effort, of the principal and supporting project participants by completing a Personnel Form. This includes the use of any consultants, subcontractors and/or vendors; provide information on this form for all such people.

Information provided on this form will automatically support subsequent forms to be completed as part of the Science PSP submission process. Please be mindful of what information you enter and how it may be represented in the Task and Budget forms.

Information regarding anticipated subcontractor services must be provided regardless if the specific service provider has been selected or not. If the specific subcontractor has not been identified or selected, please list TBD (to be determined) in the Full Name field and the anticipated service type in the Title field (example: Hydrology Expert).

Please provide this information before continuing to those forms.

May, Bernie, PhD

*This person is the **Lead Investigator**. Contact information for this person is required.*

Full Name	***May, Bernie, PhD***	example: Wright, Jeffrey R., PhD.
Institution	***University of California, Davis***	*This list comes from the project form.*
Title	***Adjunct Professor***	*example: Dean of Engineering*
Position Classification	***primary staff***	
Responsibilities	Overall project coordination and implementation, data analysis and writing	
Qualifications		*You have already uploaded a PDF file for this question. Review the file to verify that appears correctly.*
Mailing Address	***2237 Meyer Hall, Dept Animal Science, Univ of CA, One Shields Ave.***	
City	***Davis***	
State	***CA***	
ZIP	***95616***	
Business Phone	***530−754−8123***	
Mobile Phone	***none***	
E−Mail	***bpmay@ucdavis.edu***	

Describe other staff below. If you run out of spaces, submit your updates and return to this form.

Williamson, Kevin, S., PhD

Full Name	***Williamson, Kevin, S., PhD***	example: Wright, Jeffrey R., PhD. Leave blank if name not known.
Institution		*This list comes from the project form.*
Title	***Research Molecular Geneticist***	*example: Dean of Engineering*

Position Classification	***primary staff***	
Responsibilities	Overall project coordination and implementation, supervise fish rearing and genetic sample procurement, supervise genetics work, data analysis and writing	
Qualifications		***This is only required for primary staff.*** *You have already uploaded a PDF file for this question. Review the file to verify that appears correctly.*

Phillips, Ruth, PhD

Full Name	***Phillips, Ruth, PhD***	example: Wright, Jeffrey R., PhD. Leave blank if name not known.
Institution	***Washington State University, Vancouver***	*This list comes from the project form.*
Title	***Adjunct Research Professor***	*example: Dean of Engineering*
Position Classification	***primary staff***	
Responsibilities	Conduct Cytogenetics work, data analysis and writing	
Qualifications		***This is only required for primary staff.*** *You have already uploaded a PDF file for this question. Review the file to verify that appears correctly.*

Pedroia, John K.

Full Name	***Pedroia, John K.***	example: Wright, Jeffrey R., PhD. Leave blank if name not known.
Institution	***University of California, Davis***	*This list comes from the project form.*
Title	***Staff Research Associate II***	*example: Dean of Engineering*
Position Classification	***secondary staff***	
Responsibilities	Assist with rearing, perform molecular genetic analysis (genotyping) of sex, assist with necropsy of offspring from controlled breeding experiments.	
Qualifications		***This is only required for primary staff.*** *Upload a PDF version of this person's resume that is no more than five pages long. To upload a resume, use the "Browse" button to select the PDF file containing the resume.*

Conflict Of Interest

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Instructions

To help Science Program staff manage potential conflicts of interest in the review and selection process, we need some information about who will directly benefit if your proposal is funded. We need to know of individuals in the following categories:

- Applicants listed in the proposal who wrote the proposal, will be performing the tasks listed in the proposal, or who will benefit financially if the proposal is funded;
- Subcontractors listed in the proposal who will perform some tasks listed in the proposal and will benefit financially if the proposal is funded.

Applicant	University of California, Davis
Submittor	May, Bernie P
Primary Staff	May, Bernie, PhD
Primary Staff	Williamson, Kevin, S., PhD
Primary Staff	Phillips, Ruth, PhD
Secondary Staff	Pedroia, John K.

Are there other persons not listed above who helped with proposal development?
No.

If there are, provide below the list of names and organizations of all individuals not listed in the proposal who helped with proposal development along with any comments.

Tasks

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Instructions

Utilize this Task Table to delineate the tasks identified in your project description. Each task and subtask must have a number, title, brief description of the task (detailed information should be provided in the project description), timeline, list of personnel or subcontractors providing services on each specific task, and list of anticipated deliverables (where appropriate). When creating subtasks, information must be provided in a way that avoids duel presentation of supporting tasks within the overall task (i.e. avoid double counting). Information provided in the Task Table will be used to support the Budget Form. Ensuring information regarding deliverables, personnel and costs associated with subtasks are only provided once is imperative for purposes of avoiding double counting of efforts within the Budget Form.

For proposals involving multiple institutions (including subcontractors), the table must clearly state which institutions are performing which tasks and subtasks.

Task ID	Task Name	Start Month	End Month	Personnel Involved	Description	Deliverables
1	***Rearing of families and sample collection***	*1*	*9*	***May, Bernie, PhD Williamson, Kevin, S., PhD Pedroia, John K.***	Rearing of offspring from breeding experiments, collection and shipment of genetic samples to collaborator, data handeling, analysis, and reporting	Quarterly reports, presentations at one local and national conference per year, final integrated manuscripts
2	***Genetic analysis of Sex***	*6*	*12*	***May, Bernie, PhD Williamson, Kevin, S., PhD Pedroia, John K.***	Genotyping of genetic sex of offspring, necropsy	Quarterly reports, presentations at one local and national conference per year, final integrated manuscripts
3	***Cytogenetic analysis of offspring***	*5*	*9*	***May, Bernie, PhD Phillips, Ruth, PhD Pedroia, John K.***	Cytogenetic characterization of individual fish, data handeling, analysis, and reporting	Quarterly reports, presentations at one local and national conference per year, final integrated manuscripts

Budget

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Instructions

All applicants must complete a budget for each task and subtask. The Budget Form uses data entered in the Task Form, thus tasks should be entered before starting this form. Failure to complete a Budget Form for each task and/or subtask will result in removal of the application from consideration for funding.

CBDA retains the right to request additional information pertaining to the items, rates, and justification of the information presented in the Budget Form(s).

Supporting details on how costs were derived for each line item must be included in the justification section for each item. The cost detail for each item should include the individual cost calculations associated with each line item to provide the basis for determining the total amount for each budget category.

Following are guidelines for completing the justification section of this form:

Labor (Salary &Wages)

Ensure each employee and associated classification is correctly identified for each task and subtask. This information will automatically be provided once the Staff Form has been completed. Provide estimated hours and hourly rate of compensation for each position proposed in the project.

Employee Benefits

Benefits, calculated as a percentage of salaries, are contributions made by the applicant for sick leave, retirement, insurance, etc. Provide the overall benefit rate and specify benefits included in this rate for each employee classification proposed in the project.

Travel

Travel includes the cost of transportation, subsistence, and other associated costs incurred by personnel during the term of the project. Provide purpose and estimated costs for all travel. Reoccurring travel costs for a particular task or subtask may be combined into one entry. The number of trips and cost for each occurrence must be clearly represented in the justification section for reoccurring travel items of this nature.

Any reimbursement for necessary travel and per diem shall be at rates specified by the California Department of Personnel Administration for similar employees (www.dpa.ca.gov/jobinfo/statetravel.shtm).

Equipment

Equipment is classified as any item of $5,000 or more and has an expected life of three years or more. Equipment purchased in whole or in part with these grant funds must be itemized. List each piece of equipment and provide a brief description and justification for each.

Supplies

Provide a basic description and cost for expendable research supplies. Costs associated with GIS services, air photos, reports, etc. must be listed separately and have a clear justification associated with each entry. Postage, copying, phone, fax and other basic operational costs associated with each task and subtask may be combined unless the cost associated with one particular service is unusually excessive.

Subcontractor Services

Subcontractor services (Professional and Consultant services) include the total costs for any services needed by the applicant to complete the project tasks. Ensure the correct organization is entered in the Personnel Form so that it appropriately appears on the Budget Form. The applicant must provide all associated costs of all subcontractors (i.e. outside service providers) when completing this form. Applicants must be able to demonstrate that all subcontractors were selected according to an applicant's institutional requirements for the selection of subcontractors (competitive selection or sole source justification).

CBDA retains the right to request that a subcontractor provide cost estimates in writing prior to distribution of grant funds.

CBDA retains the right to request consultant, subcontractor, and/or outside service provider cost estimates in writing prior to distribution of grant funds.

Indirect Costs (Overhead)

Indirect costs are overhead expenses incurred by the applicant organization as a result of the project but are not easily identifiable with a specific project. The indirect cost rate consists of a reasonable percentage of all costs to run the agency or organization while completing the project. List the cost and items associated with indirect costs. (These items may include general office expenses such as rent, office equipment, administrative staff, operational costs, etc. Generally these items are represented by the applicant through a predetermined percentage or surcharge separate from other specific costs of items necessary to complete a specific task or subtask.)

If indirect cost rates are different for State and Federal funds, please identify each rate and the specific items included in the calculation for that rate.

Task 1, Rearing Of Families And Sample Collection: Labor	**Justification**	**Amount**
May, Bernie, PhD	***Adjunct Professor IV, 125 hr. @ $47.00/Hr.***	***5875***
Williamson, Kevin, S., PhD	***n/a***	***0***
Pedroia, John K.	***Staff Research Associate II, 750 hr. @ $18.00/Hr.***	***13500***
Task 1, Rearing Of Families And Sample Collection: Benefits	**Justification**	**Amount**
May, Bernie, PhD	***30%***	***1762***
Williamson, Kevin, S., PhD	***n/a***	***0***
Pedroia, John K.	***30%***	***4050***
Task 1, Rearing Of Families And Sample Collection: Travel Expenses	**Justification**	**Amount**
Other	***Travel for sampling of adults, performing breeding experiments, transfer of fish***	***3000***
Task 1, Rearing Of Families And Sample Collection: Supplies And Expendables	**Justification**	**Amount**
Office/Presentation Supplies	***office supplice***	***250***
Telephone	***phone fees***	***150***
Other	***Misc. lab supplies***	***1000***
Other	***Fish rearing &feeding***	***4500***
Task 1, Rearing Of Families And Sample Collection: Subcontractors	**Justification**	**Amount**
No subcontractor was assigned to this task.		
Task 1, Rearing Of Families And Sample Collection: Equipment	**Justification**	**Amount**
		0
Task 1, Rearing Of Families And Sample Collection: Other Direct	**Justification**	**Amount**
		0
Task 1, Rearing Of Families And Sample Collection: Indirect (Overhead)	**Justification**	**Amount**
	indirect costs (25%)	***8522***
	Task 1 Total	$42,609
Task 2, Genetic Analysis Of Sex: Labor	**Justification**	**Amount**
May, Bernie, PhD	***Adjunct Professor IV, 275 hr. @ $47.00/Hr.***	***12925***
Williamson, Kevin, S., PhD	***n/a***	***0***
Pedroia, John K.	***Staff Research Associate II, 700 hr. @ $18.00/Hr.***	***12600***
Task 2, Genetic Analysis Of Sex: Benefits	**Justification**	**Amount**
May, Bernie, PhD	***30%***	***3878***
Williamson, Kevin, S., PhD	***n/a***	***0***
Pedroia, John K.	***30%***	***3780***
Task 2, Genetic Analysis Of Sex: Travel Expenses	**Justification**	**Amount**
Air/Train	***Travel to Davis to perform necropsies on fish***	***1700***
Conferences	***travel to Interagency Ecological Program meetings, poster making fees***	***500***
Task 2, Genetic Analysis Of Sex: Supplies And Expendables	**Justification**	**Amount**
	Molecular supplies	***4000***

Other		
Office/Presentation Supplies	***office/presentation supplies***	***250***
Telephone	***phone fees***	***150***
Other	***publication costs***	***1000***
	Misc. lab supplies	***1000***
	equipment maintenance	***5000***
Task 2, Genetic Analysis Of Sex: Subcontractors	**Justification**	**Amount**
No subcontractor was assigned to this task.		
Task 2, Genetic Analysis Of Sex: Equipment	**Justification**	**Amount**
		0
Task 2, Genetic Analysis Of Sex: Other Direct	**Justification**	**Amount**
		0
Task 2, Genetic Analysis Of Sex: Indirect (Overhead)	**Justification**	**Amount**
	indirect costs (25%)	***11696***
	Task 2 Total	$58,479
Task 3, Cytogenetic Analysis Of Offspring: Labor	**Justification**	**Amount**
May, Bernie, PhD	***Adjunct Professor IV, 125 hr. @ $47.00/Hr.***	***5875***
Phillips, Ruth, PhD		***0***
Pedroia, John K.	***Staff Research Associate II, 200 hr. @ $18.00/Hr.***	***3600***
Task 3, Cytogenetic Analysis Of Offspring: Benefits	**Justification**	**Amount**
May, Bernie, PhD	***30%***	***1763***
Phillips, Ruth, PhD	***0***	***0***
Pedroia, John K.	***30%***	***1080***
Task 3, Cytogenetic Analysis Of Offspring: Travel Expenses	**Justification**	**Amount**
Conferences	***present research results at CALFED Sci Conference***	***400***
Conferences	***present research results at National conferences***	***1600***
Task 3, Cytogenetic Analysis Of Offspring: Supplies And Expendables	**Justification**	**Amount**
Other	***cytological reagents &supplies***	***17000***
Postage/Delivery	***shipping supplies and fees***	***1300***
	blood drawing supplies	***500***
	publication costs	***1000***
Task 3, Cytogenetic Analysis Of Offspring: Subcontractors	**Justification**	**Amount**
No subcontractor was assigned to this task.		
Task 3, Cytogenetic Analysis Of Offspring: Equipment	**Justification**	**Amount**
		0
Task 3, Cytogenetic Analysis Of Offspring: Other Direct	**Justification**	**Amount**
		0
Task 3, Cytogenetic Analysis Of Offspring: Indirect (Overhead)	**Justification**	**Amount**
	indirect costs (25%)	***8529***
	Task 3 Total	$42,647
	Grand Total	$143,735

X The indirect costs may change by more than 10% if federal funds are awarded for this proposal.

What is the total of non−federal funds requested?

ARE 'APPARENT' SEX REVERSED CHINOOK SALMON A SYMPTOM OF GENOTOXICITY?

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A. PROJECT PURPOSE

Strategic goals of the California Bay-Delta Authority (CBDA) involve the assessment of both at-risk species and water and sediment quality within the Central Valley. The objectives of the first goal (Endangered and Other At-risk Species and Native Biotic Communities) are to (1) achieve recovery and then large self-sustaining populations of at-risk native species such as Chinook salmon that depend upon the Delta, Suisun Bay and Marsh and (2) provide quantitative measures of known stressors (i.e. degraded habitat, land use). The goal regarding harvested species such as Chinook salmon is to ensure that hatchery, rearing, and planting programs do not have detrimental effects on wild populations. The main objective behind the assessment of water and sediment quality is to improve and/or maintain water and sediment quality conditions of toxic contaminants in all aquatic environments in the Bay-Delta estuary and watersheds to levels that do not adversely affect aquatic organisms, wildlife, and human health. Specific, quantified information on the effect of different stressors are key information gaps for both key species and many other Delta resources. A long-term goal of CBDA is to increase the credibility of management discussions about different up- and down-stream restoration and management actions by testing alternative hypotheses about the relative importance of different stressors, such as chemical contaminants, to the persistence of at-risk species. This study plan will undertake targeted research to provide information for future adaptive management of Chinook salmon in the Sacramento and San Joaquin River basins. We are applying for support for a collaborative project designed to address this primary data gap for Chinook salmon. Our approach will be to use molecular genetic techniques coupled with previously performed controlled breeding experiments to determine the chromosomal mechanism responsible for producing 'apparent' XY-female fall-run Chinook salmon.

B. PROJECT DESCRIPTION

B.1 Objectives and specific hypotheses

The primary goal of this research project is to test hypotheses to aid in the implementation of the CALFED Ecosystem Restoration goal for fall-run Chinook salmon with respect to the relative importance of chemical stressors on population viability and genetic diversity. Testing these hypotheses will also aid in reducing uncertainty regarding the negative impact that reproduction by 'apparent' sex-reversed individuals have on populations of this at-risk species. We will apply molecular genetic methodology to elucidate the chromosomal mechanism responsible for producing 'apparent' XY-female fall-run Chinook salmon that have been observed in California waterways (Williamson and May 2002). Cytogenetic analyses, evaluation of genotypic sex, and necropsies to ascertain gonad phenotype will be performed on offspring produced by phenotypic female, fall-run Chinook salmon that test positive for Y-chromosome specific markers ('apparent' XY-females) and those that test negative for the markers (genetically normal females). We will address the following hypotheses:

1. Is the 'apparent' sex-reversed phenotype observed in fall-run Chinook salmon due to a Y- to X-chromosome/autosome translocation?
    a. H_o: Male & female offspring of apparent XY-female fall-run Chinook salmon produce patterns of chromosome staining that are consistent with recombination of the male (Y-chromosome) specific markers to the X-chromosome/autosome.

2. Is the 'apparent sex-reversed' phenotype observed in fall-run Chinook salmon due to a Y-chromosome that has had the sex determining region deleted or inactivated?
    a. H_o: Male & female offspring of apparent XY-female fall-run Chinook salmon produce patterns of chromosome staining that are consistent with a Y-chromosome that lacks a functional sex-determining region.

3. Does the OtY2(WSU) Y-chromosome specific marker exhibit the same pattern of inheritance as two other male-specific markers, OtY1 and the growth hormone pseudogene, in a controlled mating between an 'apparent' XY-female and a genetically normal male?
    a. H_o: The Y-chromosome specific marker OtY2(WSU) is inherited in a manner consistent with Mendelian segregation. Specifically, does a controlled cross between a normal male (XY) and an 'apparent' XY-female produce a 1:3, female to male, genotypic sex ratio in the offspring?

B.2 Background information and conceptual models

The phenomenon of 'apparent' sex-reversal of male, fall-run Chinook salmon is due to a heritable mutation rather than altered sexual differentiation during early development (Williamson and May, *in submission*). Since sex is chromosomally determined in Pacific salmon (Thorgaard 1977; Thorgaard and Gall 1979, Ueda an Ojima 1984a,b), mutational changes to the sex chromosomes may have a profound impact on the expression of sexual characteristics, development, and function. Controlled breeding experiments conducted by our lab in 2003 have shown that approximately half of the phenotypic female offspring of XY-females have a male genotype according to two Y-chromosome specific markers, OtY1 (Devlin et al. 1991, 1994) and the growth hormone pseudogene (GH-Ψ) (Du et al. 1993). The data present strong evidence that phenotypic female Chinook salmon with a male genotype (XY-females) are the result of a genetic mutation rather than altered sexual differentiation. Either the OtY1 and GH-Ψ markers have recombined on the sex chromosomes (a Y-chromosome to autosome translocation is also possible), or a mutation has either eliminated or inactivated the sex-determining region of the Y-chromosome. If, as was hypothesized by Williamson and May (2002), apparent XY-females were the result of altered sexual differentiation, owing to exposure to environmental endocrine disrupting chemicals (EDCs), one would not expect to observe incongruent sexual genotype and phenotype within individual progeny of these fish. If the developmental pathway(s) controlling sexual phenotype were altered by exposure to EDCs at a critical period of development such changes may occur without

necessarily also eliciting a heritable change in the germ cells of the exposed individuals. Despite the strong evidence against altered sexual differentiation due to exposure to EDCs, uncertainty exists regarding the nature of the chromosomal mechanism responsible for producing 'apparent' XY-female fall Chinook salmon, how the mutation originally arose, and the potential threat that reproduction by XY-females poses to the population persistence.

Chemical compounds that are genotoxic can cause population reduction by the effects of somatic and heritable mutations, as well as non-genetic modes of toxicity (Bickman et al. 2000). These compounds may form adducts directly with DNA, create breaks or lesions on DNA strands, or interfere with the cellular DNA repair mechanisms that eliminate both lesions to, or adducts that form on DNA strands. Should such damage accumulate within the genome, multiple cellular functions may be negatively impacted. Genotoxicity can lead to aberrant regulation, expression, or complete elimination of gene products. In turn, the cellular processes and the physiological functions that they control would be negatively impacted. Stochastic processes in small populations, increased mutation load, and the phenomenon of mutational meltdown are synergistic factors that cause reduced fitness and accelerate the process of population extirpation. Although the original damage caused by chemical contaminants is at the molecular level, there are emergent effects at the population level, such as the loss of genetic diversity (Bickman et al. 2000). Indeed, genetic changes may be the precursors of some of the numerous effects reported at higher levels of biological organization such as the feminization of males, developmental abnormalities, and infertility (Atzar et al. 2002). Problems such as these would contribute to the loss of genetic variation within and decreased reproductive potential of affected populations.

DNA damage is not necessarily limited to the individual in which the damage originally occurs. DNA damage that occurs in the progenitor cells of gametes may be inherited by subsequent generations if that damage is not corrected by cellular repair mechanisms. Incorrect repair of damaged DNA may also lead to incorrect pairing of chromosomes during meiosis thereby producing gametes without a complete set of genetic information. In this respect, chronic exposure to genotoxic substances can, over time, have a progressively more negative effect on exposed populations.

If a single genetic locus determines sexual phenotype, the adjacent chromosome region where that locus resides will be restricted to the heterogametic sex (i.e.- Y-chromosome in males) (Devlin et al. 2001). Consequently the Y-chromosome cannot become homozygous in individuals within a population, which decreases the ability of selective pressures to remove recessive deleterious rearrangements or mutations in this region (Lucchesi 1978; Charlesworth 1991). This permits opportunity for genetic alterations to accumulate over time on the Y-chromosome. Mutations within or physically near the sex-determining region may increase in frequency in a population through evolutionary mechanisms such as reduced recombination, Muller's ratchet (Muller 1964, Felsenstein 1974), hitchhiking with high fitness alleles, or dosage compensation (Muller 1932; Lucchesi 1978; Charlesworth 1996).

Apparent sex-reversal of male, fall-run Chinook salmon may be symptomatic of exposure of fish to genotoxic environmental contaminants. Mutational degradation of the Y-chromosome and subsequent loss of the sex determining region, or incorrect repair of damage to the Y-chromosome leading to a translocation between the sex chromosomes may be responsible for the 'apparent' XY-female fall Chinook observed in California Rivers. Chronic and/or acute exposure to chemical contaminants at any life history stage can lead to DNA damage. Such damage would occur genome-wide, and it may accumulate over time near the sex-determining region on the Y-chromosome due to the decreased ability of selective pressures to remove mutational changes in this region. It is possible that mutations due to DNA damage that have accumulated on the Y-chromosome over successive generations would eventually degrade its' integrity the point where the sex-determining locus itself was either inactivated, deleted, or during the process of DNA repair a portion of the Y-chromosome bearing Y-specific markers had inadvertently been translocated to the X-chromosome or an autosome. The events that produce 'apparent' sex-reversed individuals do not appear to be temporally or geographically limited within California's Central Valley (Williamson and May 2002, *in submission*). Frequent, true sex-reversal, as would occur by the presence of a Y-chromosome that lacks a functional sex-determining locus, could have long-term serious implications for genetic diversity a species (Devlin et al. 2004, Williamson and May 2002). Conversely, if male-specific genetic markers have merely moved to the X chromsome or an autosome, and are thus no longer exclusively associated with the Y chromosome, apparent sex-reversal through such a mechanism may pose no serious threat to Chinook salmon populations (Devlin et al. 2004).

Artificial families created through controlled breeding of genetically normal and apparent XY-female fall-run Chinook salmon would provide a tool that will allow us to determine the chromosomal mechanism responsible for the observed incongruence between genotypic and phenotypic sex. By performing Fluorescent In Situ Hybridization (FISH) to examine how the Y-chromosome specific probes OtY1 and GH-Ψ are localized on chromosomes of the offspring produced from artificial families one can discriminate between alternate models (Figure 1) used to explain the incongruence between sexual genotype and phenotype of fall-run fish. Uncovering the chromosomal mechanism responsible for producing apparent XY-female Chinook salmon would provide a better understanding of the exact molecular genetic change(s) that occur thereby producing apparent XY-females, whether or not reproduction by these fish is a stressor to population persistence, and if the presence of XY-females is symptomatic of genotoxicity experienced by California Chinook salmon populations. If reproduction by XY-female Chinook salmon poses a risk to population persistence, then keeping XY-females out of hatchery programs may be required to prevent accelerated deterioration of genetic diversity of this harvested species. Furthermore, information provided by our proposed research project can lead to the mapping of and subsequent characterization of regulation and expression of the sex-determining locus on the Chinook salmon Y-chromosome itself.

Performing Fluorescent In Situ Hybridization with the previously developed OtY1 and growth hormone pseudogene probes (Devlin et al. 1991, 1994; Du et al. 1993,

respectively) can be used to determine if apparent XY-females carry either a translocation between the Y- and X-chromosome/autosome, or a Y-chromosome that lacks a functional sex-determining locus. Chromosome spreads may be obtained from the offspring of phenotypic females positive for both Y-chromosome markers or genetically normal, phenotypic females using nucleated blood cells (lymphocytes). By comparing the pattern of fluorescent chromosome staining produced by the progeny from both genetically normal and apparent XY-females, the FISH assay would reveal which of the two mutational scenarios is responsible for the observed incongruence between sexual genotype and phenotype. This assumes that the FISH assay can provide sufficient resolution to differentiate between the patterns of chromosome fluorescent staining produced by a Y- to X-chromosome/autosome translocation and an intact, normal Y-chromosome. Offspring produced from the cross between genetically normal, phenotypic females and males (control assay) would exhibit a bright pattern of chromosomal staining with the OtY1 probe localized at the distal end of the short arm of an acrocentric chromosome only in phenotypic males (Stein et al. 2001). Phenotypic female offspring from the control cross do not exhibit the bright, localized fluorescent staining that is observed in males.

Evidence that a phenotypic female positive for both Y-chromosome markers passed on a Y- to X-chromosome/autosome translocation (X^m) would be revealed by roughly half of its phenotypic female offspring (XX^m) exhibiting a positive, albeit different, pattern of chromosome fluorescent staining from males of the control cross as well as their own male siblings that either did or did not receive the translocation (X^mY or XY, respectively, in figure 2A). Individual phenotypic male offspring that received the translocation (X^mY in figure 2A) would exhibit fluorescent staining on two separate chromosomes, the translocated X^m and its unaffected Y-chromosome, These phenotypic male offspring would exhibit a pattern of chromosome staining that overlapped both the patterns obtained from their affected phenotypic female (XX^m) and unaffected phenotypic male (XY) siblings.

Evidence that an apparent XY female parent passed on a Y-chromosome lacking a functional sex-determining locus (Y^F) should be revealed by a similar FISH pattern between its phenotypic male offspring that did not receive the mutation, their phenotypic female siblings that did (XY and XY^F, respectively, in figure 2B), and the phenotypic males of a control cross. Additionally, phenotypic male offspring that receive the Y^F-chromosome (YY^F in figure 2B) should exhibit a pattern of staining on two separate chromosomes similar to that observed on the single Y-chromosome of phenotypic male offspring of the control cross. It is possible that a nonsense, or a small insertion-deletion mutation within the sex-determining locus is responsible for the apparent sex-reversed phenotype. In either of these cases, the mutational change itself would not create a structural change in the chromosome that is large enough to be resolved by the FISH assay. In such a case it may not be possible to discriminate between a wild-type (normal) and a Y-chromosome lacking a functional sex-determining locus using this *in-situ* staining method.

B.3 General scope of work

Fish Rearing Activities

We have successfully performed controlled breeding experiments using genetically normal and apparent XY-female fall-run Chinook salmon during the last three spawning seasons (2002 to 2004). Once additional families have been reared to the pre-smolt stage of development we will have sufficient material with which to test hypotheses. The control and experimental families of fish produced from genetically normal females and XY-females, respectively, will be reared at the Center for Aquatic Biodiversity and Aquaculture (UC Davis).

Sample collection for artificial crosses– Fin-clips for genetic analysis and gametes from fall-run Chinook salmon returning to the Merced River Fish Hatchery (MRH) will be collected with the assistance of California Department of Fish and Game personnel during the spawning period (Late October to Early December) of these fish. . Approximately 2 cm^2 of caudal fin tissue near the caudal peduncle will be excised with scissors from each fish sampled and placed into separate, labeled coin envelopes. Eggs from phenotypic females will be expressed into pre-labeled plastic urine analysis cups, sealed and immediately placed on a raised platform within an ice chest. Milt from three phenotypic males will be expressed into labeled Zip-Loc® bags and similarly stored. Tissue samples and gametes will be stored between 5-8^oC while transported back to the University of California Davis Genomic Variation Lab (GVL) for genetic analysis and use in controlled breeding experiments conducted at the Center for Aquatic Biodiversity and Aquaculture (CABA), respectively.

Genetic screening to detect apparent sex-reversed male (XY female) fish – The selection criterion for sets of gametes to be used in artificial crosses will be based on the sexual genotype at the Growth Hormone pseudogene and OtY1 loci of putative parents. All phenotypic female and male fall-run Chinook sampled will be genetically screened by polymerase chain reaction (PCR) assays using the OtY1 primers developed from Chinook salmon by Devlin et al. (1994) and the Growth Hormone pseudogene (GH-Ψ) primers developed by Du et al. (1993). DNA fragments amplified by PCR were resolved on a 5.5% acrylamide-7M Urea gel and imaged by a MJ Research BaseStation (MJ Research, San Francisco, California). Individual genotypes will be scored using Cartographer® software as well as manually verified for every individual genotyped.

Chinook that test positive for a 209 base pairs (bp) PCR fragment and that did not produce a series of larger PCR products characteristic of the OtY1 locus in females (Devlin et al. 1994) and that tested positive for a 276 bp band indicative of the GH Ψ (Du et al. 1993) were scored as being positive for having the Y-chromosome markers (genetic males). In the case where a fish that had ovaries produced both a robust 209 bp PCR fragment (OtY1) and the 276 bp fragment (GH-Ψ), that fish was scored as a XY-female. When the larger PCR fragments characteristic of females were present and the 209 bp PCR fragment was not present and the 276 bp band indicative of the GH pseudogene was also absent, the fish was scored as being negative for having the Y-chromosome markers (genetic female).

Breeding experiments - The eggs from each single phenotypic female fish selected will be split into roughly two equal portions and placed into separate styro-foam containers. Aliquots of eggs will be separately fertilized with milt from separate, single, genetically normal males. In this manner, each family of fish will have only two parents. Hatchlings from individual families will be incubated at 12^{o}C for approximately 45 days (just before swim up stage) in Heath trays and will be transferred to separate, larger rearing tanks.

Collection of genetic samples from offspring - Once development has proceeded to the pre-smolt stage, whole blood will be drawn via caudal vein puncture into sterile, heparinized collection tubes from euthanized individuals using standard procedures (Phillips 2005). Whole blood from separate individuals will be shipped overnight to Dr. Ruth Phillips (participating investigator) for cytogenetic analysis. During blood collection for each individual a tissue sample will be taken for analysis of genetic sex, and a necropsy will be performed to ascertain gross gonad morphology. Genetic sex of each individual will be evaluated (as above) using the Y-chromosome specific probes OtY1 and growth hormone pseudogene as well as another Y-chromosome marker OtY2(WSU) recently developed by Brunelli and Thorgaard (2004). Since there is evidence that OtY2(WSU) is not in physical proximity to either of the other two Y-chromosome specific probes in Chinook salmon (Brunelli and Thorgaard 2004) we have decided to incorporate this new marker for genetic sex into our analyses. It is possible that the pattern of inheritance of the OtY2(WSU) locus differs from the other two in families produced by 'apparent' XY-female fall Chinook salmon. Using the additional marker may provide valuable insight into the chromosomal mechanism responsible for 'apparent' sex-reversal of Chinook salmon.

Cytogenetic analysis of individuals

Metaphase chromosome preparations will be made from lymphocyte cultures using standard procedures (Phillips 2005). The Y-chromosome will be identified using either a plasmid clone containing OtY8 cosmid and or a cosmid clone containing the GH-Y gene from Chinook salmon (provided by Robert Devlin, Fisheries and Oceans Canada) or both. High molecular weight DNA will be extracted from the plasmid using standard procedures and from the cosmid using a Qiagen kit. Fluorescence in situ hybridization (FISH) analysis will be done as described in Stein et al., (2001) with minor modifications. The clones will be labeled either with Spectrum Orange (Vysis, Inc.) or digoxigenin (Roche, Inc.). Blocking repetitive sequence (Cot1 DNA) is added to the probe and the probe and chromosomal DNA are thermally denatured. The denatured probe is added to the slide that is hybridized overnight under controlled stringency. After a series of post-hybridization washes, slides are counterstained with DAPI and viewed under fluorescent illumination. For digoxigenin labeled probes, antibodies to digoxigenin (Roche, Inc.) diluted in phosphate buffered saline (PBS) are added to the slides after the post-hybridization washes and the slides are incubated for 45 minutes before a final series of washes and application of the DAPI counter stain. Fluorescent images are captured

separately for each fluorochrome with a digital camera and combined using the CYTOVISION image analysis program (Applied Imaging, Inc).

C. PROJECT JUSTIFICATION

C.1 Relevance to CBDA management

The study of genetic mechanisms that alter sexual development of Central Valley Chinook salmon is within the CALFED Program goals of protecting and improving At-Risk Native Species populations and the CALFED Science Program of addressing the uncertainties used for management and developing performance measures. We anticipate that the results of this study will provide relevant information on the possible impact of reproduction by 'apparent' XY-female Chinook salmon have on genetic diversity of populations in which they occur at high frequency. We believe that the results of this study will provide critical information to the scientific community, CALFED Agency managers and stakeholders on salmonid restoration and management issues in San Joaquin River and Sacramento River watersheds. Our project will contribute information to the following specific Priority Topic Areas:

- Life cycle models and population biology of target species
  This research will contribute to the knowledge base about the basic biology of Chinook salmon that could be helpful for long-term management. This information can be incorporated with other population-level data used to refine conceptual life cycle models and develop relevant credible management strategies.
- Relative stresses on target fish species
  Our proposed research will address knowledge gaps regarding the impact that reproduction by 'apparent' XY-female Chinook salmon has on the population genetics of fall-run populations in California. Furthermore, elucidating the chromosomal mechanism responsible for producing the 'sex-reversed' phenotype observed in fall Chinook can indicate whether or not XY-female Chinook is a symptom of genotoxicity due to exposure of fish to environmental contaminants.

C.2 Feasibility, capability, and collaboration

The research outlined in this proposal is not dependent on the outcomes of other projects, and (generally) independent of natural conditions (i.e. weather). The project requires no CEQA, NEPA, or other environmental compliance documents. Both a scientific collection permit (CDFG), and an Animal Use and Care Protocol (#04-11435, accepted 12/2004, UC Davis) have been obtained. UC Davis has the appropriate molecular genetic laboratories and fish rearing facilities required for this project. No zoning regulations, planning ordinances or other constraints that could impact the schedule and the ability to implement the project are known.

The research project will involve neither physical alterations (i.e. grading, planting vegetation, or breeching levees) to land, public or private, nor restrictions in land use (i.e. conservation easement or placement of land in a wildlife refuge). The collection of fish specimens will not require access to private property.

The members of the research team have considerable skill and experience with all of the proposed molecular methodology and fish rearing practices. Each member has demonstrated the ability to (1) successfully collaborate with individuals from other agencies or organizations on interdisciplinary projects, and (2) publish results in scholarly journals. The primary responsibilities for each research team member are provided in Table 1.

C.3 Dissemination of results

Quarterly reports will include financial status, activities during the quarter, tasks completed, and deliverable items produced, problems encountered, and how those problems were overcome. A final technical report describing results of the study will be submitted at the end of the project. Results of this study will be presented at scientific (CALFED Science Conference) and technical meetings (Interagency Ecological Program). In addition, results of this study will be submitted as manuscripts for publication in peer-reviewed scientific journals. The Principal Investigator will store all data for a minimum of five years after project completion. The overall project outcome will be to develop a better understanding of the chromosomal mechanism responsible for the 'apparent' sex-reversal phenotype observed in fall-run Chinook salmon in the Sacramento and San Joaquin drainages. This base of knowledge will be useful for researchers and natural resource decision makers requiring information regarding the effect of environmental contaminants on the reproduction function and population persistence of this of this at-risk species.

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Table 1. Division of primary responsibilities among research team members.

Researcher	Role	Primary responsibilities
May	applicant and principal investigator	Overall project coordination and implementation, data analysis and writing
Williamson	principal investigator	Overall project coordination and implementation, supervise fish rearing and genetic sample procurement, supervise genetics work, data analysis and writing
Phillips	participating investigator	Conduct Cytogenetics work, data analysis and writing

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A.

Phenotypic female scored as genotypic male at both OtY1 & GH-psi

Phenotypic male scored as genotypic male at both OtY1 & GH-psi	**X**	**X^m**
X	**female** X X	**female** X^m X
Y	**male** X Y	**male** X^m Y

B.

Phenotypic female scored as genotypic male at both OtY1 & GH-psi

Phenotypic male scored as genotypic male at both OtY1 & GH-psi	**X**	**Y^F**
X	**female** X X	**female** X Y^F
Y	**male** X Y	**male** YY^F

Figure 1 - Alternate models to explain incongruence of genotypic and phenotypic sex in half of the phenotypic female offspring produced by apparent XY-female fall-run Chinook salmon. An X-chromosome carrying a translocated portion of the Y-chromosome designated by X^m (A), and a Y-chromosome lacking the sex-determining region designated by Y^F (B) from the phenotypic female parent are shown. Both models predict both the 1:1, male to female, phenotypic, and the 3:1, male to female, genotypic sex ratios observed in offspring from XY-females used in breeding experiments. Wild type X- & Y-chromosomes designated by X and Y, respectively. Sexual phenotype of offspring is in bold. Taken from Williamson and May (2002).

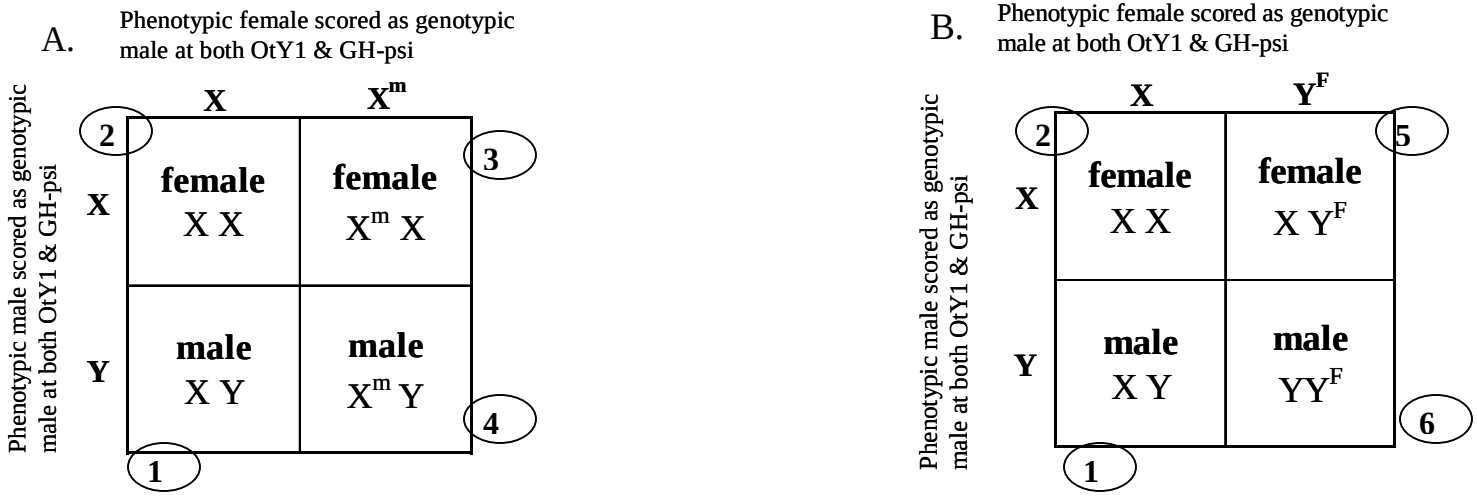

Figure 2 - The patterns of fluorescent staining on the chromosomes of offspring, using the Y-chromosome specific probes OtY1 & growth hormone pseudogene, may differentiate between which of the two proposed chromosomal mechanisms is responsible for producing 'apparent' XY-female Chinook observed in California rivers.

1) Unaffected male offspring (XY) – intense, localized staining on the single Y-chromosome present in a pattern similar to that produced by a phenotypic male in a control family;
2) Unaffected female offspring (XX) - no localized staining on a chromosome since there's no Y-chromosome present;
3) Affected female offspring (XX^m) - staining on a single chromosome in a pattern different from that produced by a phenotypic male in a control family since a translocation would carry only a portion of the Y-chromosome;
4) Affected male offspring (X^mY) - two chromosomes are fluorescently stained, one similar to that observed in 1, the other similar to that in 3;
5) Affected female offspring (XY^F) - intense, localized staining on a single chromosome in a pattern similar to that produced by a phenotypic male in a control family;
6) Affected male offspring (YY^F) - intense, localized staining on two chromosomes each in a pattern similar to that produced by a phenotypic male in a control family.

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EDUCATION

Ph.D., The Pennsylvania State University, in Genetics--1980
"The salmonid genome: evolutionary restructuring following a tetraploid event."
M.S., University of Washington, in Fisheries--1975
"Electrophoretic variation in the genus *Oncorhynchus*: the methodology, genetic basis, and practical applications to fisheries research and management."
B.S., University of Washington, in Molecular Biology--1973

EMPLOYMENT

1995 to pres.	Adjunct Professor, Director, Genomic Variation Laboratory, Department of Animal Science, Univ. of California, Davis, CA 95616.
1992-1995	Senior Research Associate and Director, Genome Variation Analysis Facility, Dept. Natural Resources, Fernow Hall, Cornell University.
1988-1992	Senior Research Associate (SRA II in 1989) and Director, CLEEG, Dept. Natural Resources, Cornell University
1981-1988	Research Associate (SRA in 1985) and Director, The Cornell Laboratory for Ecological and Evolutionary Genetics (CLEEG), Section of Ecology and Systematics, Cornell University
1980-1981	Research Associate, Department of Plant Pathology,The Pennsylvania State University

RESEARCH INTERESTS

My research over the past three decades has centered around the use of discrete Mendelian data to answer a broad array of biological questions in fungi, fish, plants, invertebrates, birds, and mammals. One of my primary roles has been to provide a genetics perspective to the collaborative projects with which I have been involved. During the past 10 years my laboratory has come to focus primarily on questions in conservation biology regarding the "genetic health" and "genetic integrity" of natural populations of threatened and endangered fish species. Examples of these questions include: How do we identify populations for preservation?, How do we measure loss of genetic variability?, What remnants of native populations remain after extensive stocking with non-indigenous populations?, How different must two populations be for them to be maintained and managed separately? My program also includes a major emphasis on mapping QTLs in aquaculture species and some effort devoted to the use of AFLPs and microsatellites to detect the effects of toxicants on the gene pools of indigenous species. I am currently developing the use of expression technologies as a more suitable technology to examine the effects of toxicants and disease.

GRADUATE STUDENT THESIS COMMITTEES

NAME	GRAD. GROUP	DEGREE	YEAR (expected)
M. Bagley	Genetics	PhD	1997
M. Brown	Ecology	MS	1998
J. Agresti	Genetics*	MS	1999
E. McQuown	Animal Science*	MS	2000
G. Tranah	Ecology*	PhD	2001
L. Daniels	Genetics	MS	2001
J. Rodzen	Genetics*	PhD	2001
N. Belfiore	Ecology*	PhD	2001
H. Ernest	Ecology	PhD	2001
S. Blankenship	Genetics	PhD	2001
K. Bucklin	Genetics	PhD	2002
B. Sacks	Ecology	PhD	2002
J. Beyer	Genetics*	MS	2002
A. Fowler	Ecology	PhD	2002
A. Whitehead	Pharm. & Tox.	PhD	2002
F. Rodriguez	Genetics*	PhD	2003
C. Floyd	Ecology	PhD	2003
E. Meredith	Genetics*	MS	2004
Z. Hogan	Ecology	PhD	2004
K. Williamson	Ecology*	PhD	2004
K. Rodrigue	Animal Science	MS	2004
C. Conway	Ecology*	PhD	(2005)
M. Teglas	Comp. Path.	PhD	(2005)
T. O'Hare	Animal Science	MS	(2005)
S. Ostermann	Ecology	PhD	(2005)
K. Coykendall	Genetics*	PhD	(2005)
R. Topinka	Ecology*	PhD	(2005)
Y. Chen	Ecology*	PhD	(2005)
J. Israel	Ecology*	PhD	(2006)
W. Savage	Ecology	PhD	(2006)
A. Welsh	Ecology*	PhD	(2006)
M. Stephens	Ecology*	PhD	(2006)
R. Robles	Ecology	PhD	(2007)
M. Baerwald	Genetics*	PhD	(2007)
R. Schwartz	Ecology*	PhD	(2008)
J. Hull	Ecology*	PhD	(2008)
J. Petersen	Genetics*	PhD	(2009)

* - Chair

REVIEWER FOR (recent)

AAAS
Biotechniques
Cons. Genet.
J. of Great Lakes Research
J. of Hered. (assoc. ed. 1997 to 2001)
Mol. Ecol.
N. Amer. J. Fish. Science
National Science Foundation
Sea Grant (Panel Chair)
USDA Competitive Research Grants

PROFESSIONAL SOCIETIES

AAAS American Fisheries Society

SCIENTIFIC ARTICLES (PUBLISHED AND IN PRESS) (last five years only)

Bayard de Volo, S., RT Reynolds, JR Topinka, B. May and MF Antolin. **In press.** Population genetic structure and the utility of muliti-locus genotyping for mark-recapture studies of northern goshawks (*Accipiter gentilis*). J. Raptor Research.

Cordes, J.F., D.L. Perkins, H.L.Kincaid, and B. May. **In press.** Genetic analysis of fish genomes and populations: allozyme variation within and among Atlantic salmon (*Salmo salar* L.) from downeast rivers of Maine. J. Fish Biology.

Leunda, P.M., R. Miranda, J. Madoz, S. Parmenter, Y. Chen, and B. May. **In press.** Threatened fishes of the world: *Siphateles bicolor snyderi* (Miller, 1973) (Cyprinidae). Env. Biol. Fish.

Teglas, M.B., B. May, P.R. Crosbie, M.R. Stephens, and W.M. Boyce. **In press.** Intraspecific variation in the 16S rDNA mitochondrial gene of *Ornithodoros coriaceus* (Acarina: Argasidae) populations from California, Oregon and Nevada. J. Med. Entomol.

Williamson, K. and B. May. **In press.** Homogenization of fall-run chinook salmon gene pools in the Central Valley of California, USA. N. Am. J. Fish. Man.

Baerwald, M.R. and B. May. **2004.** Characterization of microsatellite loci for five members of the minnow family Cyprinidae found in the Sacramento - San Joaquin Delta. Mol. Ecol. Notes. 4:385-390.

Cordes, J.F., J.A. Israel and B. May. **2004.** Conservation of Paiute cutthroat: the genetic legacy of popultion transplants in an endemic California salmonid. CA Fish and Game. 90-101-118.

Flores-Martínez, J.J., C. Floyd, L.G. Herrera, and B. May. **2004.** Genetic variation and population size in the fishing bat, *Myotis vivesi*, in Isla Partida. In: Homenaje a Bernardo Villa, Eds., R. Medellín and V. Sánchez-Cordero. Publ. Universidad Nacional Autónoma de México. Pp. 185-190.

Hogan,Z.S., P.B. Moyle, B. May, M.J. Vander Zanden and I.G. Baird. **2004.** Imperiled Giants of the Mekong: Ecologists struggle to understand—and protect—Southeast Asia's large migratory catfish. Am. Sci. 92:228-237.

Israel, J.A., J.F. Cordes, M.A. Blumberg, and B. May. **2004.** Geographic patterns of genetic differentiation among collections of green sturgeon. N. Am. J. Fish. Man. 24:922-931.

Moen, T., J.J. Agresti, A. Cnaani, H. Moses, T.R. Famula, G. Hulata, G.A.E. Gall, B. May. **2004.** A genome scan of a four-way tilapia cross supports the existence of a quantitative trait locus for cold tolerance on linkage group 23. Aqua. Res. 35:893-904.

Rodriguez, F., S. LaPatra, S. Williams, T. Famula and B. May. **2004.** Genetic markers associated with resistance to infectious hematopoietic necrosis in rainbow and steelhead trout (*Oncorhynchus mykiss*) backcrosses. Aquaculture 241:93-115.

Rodzen, J.A., T. Famula, and B. May. **2004.** Estimation of parentage and relatedness in the polyploid white sturgeon *Acipenser transmontanus* using a dominant marker approach for duplicated microsatellite loci. Aquaculture 232:165-182.

Schwartz, R.S. and B. May. **2004.** Characterization of microsatellite loci in Sacramento perch (*Archoplites interruptus*). Mol. Ecol. Notes. 4:694-697.

Stadelmann, B., L.G. Herrera, J. Arroyo-Cabrales, J.J. Flores-Martínez, B.P. May, and M. Ruedi. **2004.** Molecular systematics of the fishing bat *Myotis (Pizonyz) vivesi*. J. Mammo. 85:133–139.

Topinka, J.R., A.J. Donovan, and B. May. **2004.** Characterization of microsatellite loci in Kearney's bluestar (*Amsonia kearneyana*) and cross-amplification in other *Amsonia* species. Mol. Ecol. Notes. 4:710-712.

Topinka, J.R. and B. May. **2004.** Development of polymorphic microsatellite loci in the northern goshawk (*Accipiter gentilis atricapillus*) and cross-amplification in other raptor species. Cons. Gen. 5:861-864.

Tranah, G.J., D.E. Campton and B. May. **2004.** Genetic evidence for hybridization of pallid and shovelnose sturgeon. J. Hered. 95:474-480.

Beyer, J. and B. May. **2003.** A graph-theoretic approach to the partition of individuals into full-sib families. Mol. Ecol. 12:2243-2250.

Ernest, H., W.M. Boyce, V. Bleich, B. May, S. Stiver, and S. Torres. **2003.** Genetic structure of mountain lion (*Puma concolor*) populations in California. Cons. Gen. 3:353-366.

May, B. **2003.** Allozyme variation. In: Population Genetics: Principles and Applications for Fisheries Scientists, Ed. E. Hallerman. Amer. Fish. Soc. Pp. 23-36.

McQuown, E., C.C. Krueger, H.L. Kincaid, G.A.E. Gall, and B. May. **2003.** Genetic comparison of lake sturgeon (*Acipenser fulvescens*) populations: differentiation based on allelic frequencies at seven microsatellite loci. J. Great Lakes Res. 29:3-13.

Tranah, G.J., M.J. Bagley, J.J. Agresti, and B. May. **2003.** Development of codominant markers for identifying species hybrids. Cons. Gen. 4:537-541.

Welsh, A.B., M. Blumberg, and B. May. **2003.** Identification of microsatellite loci in lake sturgeon, *Acipenser fulvescens*, and their variability in green sturgeon, *A. medirostris*. Mol. Ecol. Notes 3:47-55.

Whitehead, A., S.L. Anderson, K.M Kuivila, J.L. Roach, and B. May. **2003.** Genetic variation among interconnected populations of *Catostomus occidentalis*: implications for distinguishing impacts of contaminants from biogeographic structuring. Mol. Ecol. 12: 2817-2833.

Blankenship, S.M., B. May, and D. Hedgecock. **2002.** Evolution of a perfect simple-sequence-repeat locus in the context of its flanking sequence. Mol. Biol. Evol. 19:1943-1951.

Hogan, Z. and B. May. **2002.** Twenty-seven new microsatellites for the migratory Asian catfish family Pangasiidae. Mol. Ecol. Notes. 2:38-41.

McQuown, E., G.A.E. Gall, and B. May. **2002.** Characterization and inheritance of six microsatellite loci in lake sturgeon (*Acipenser fulvescens*). Trans. Am. Fish. Soc. 131:299-307.

Meredith, E.P. and B. May. **2002.** Microsatellite loci in the Lahontan tui chub, *Gila biocolor obesa*, and their utililization in other chub species. Mol. Ecol. Notes. 2:156-158.

Rodzen, J.A. and B. May. **2002.** Inheritance of microsatellite loci in the white sturgeon (*Acipenser transmontanus*). Genome 45:1064-1076.

Smith, C.T., R.J. Nelson, S. Pollard, E. Rubridge, S.J. McKay, J. Rodzen, B. May, and B. Koop. **2002.** Population genetic analysis of white sturgeon (*Acipenser transmontanus*) in the Fraser River. J. Appl. Icthy. 18:307-312.

Williamson, K.S., J.F. Cordes, and B. May. **2002.** Characterization of microsatellite loci in Chinook salmon (*Oncorhynchus tshawytscha*) and cross-species amplification in other salmonids. Mol. Ecol. Notes. 2:17-19.

Williamson, K. and B. May. **2002.** Incidence of phenotypic female chinook salmon (*Oncorhynchus tshawytscha*) positive for the Y-chromosome specific marker OtY1 in the Central Valley. J. Aquat. An. Health. 176-183.

Zhu, B., F. Zhou, H. Cao, Z. Shao, N. Zhao, B. May, and J. Chang. **2002.** Analysis of genetic variation in the Chinese sturgeon, *Acipenser sinensis*: estimating the contribution of artificially produced larvae in a wild population J. Appl. Icthy. 18:301-306.

Bagley, M.J., S. Anderson, and B. May. **2001.** Choice of methodology for assessing genetic impacts of environmental stressors: polymorphism and reproducibility of RAPD and AFLP fingerprints. Ecotoxicology 10:239-244.

Jenneckens I., J.-N. Meyer, G. Hörstgen-Schwark, B. May, L. Debus, A. Ludwig. **2001.** A fixed allele at microsatellite LS-39 exhibiting species-specificity for black caviar producer *Acipenser stellatus*. J. Appl. Icthyol. 17:39-42.

Pyatskowit, J., C.C. Krueger, H.L. Kincaid, and B. May. **2001.** Inheritance of microsatellite loci in the polyploid derivative lake sturgeon (*Acipenser fulvescens*). Genome 44:185-191.

Tranah, G.J., J.J. Agresti, and B. May. **2001.** New microsatellite loci for suckers (Catostomidae): primer homology in *Catostomus*, *Chasmistes*, and *Deltistes*. Mol. Ecol. Notes 1:55-60.

Tranah, G.J., H.L. Kincaid, C.C. Krueger, D.E. Campton, B. May. **2001.** Reproductive isolation in sympatric populations of pallid and shovelnose sturgeon. N. Am. J. Fish. Man. 21:367-373.

Agresti, J.J., S. Seki, A. Cnaani, S. Poompuang, E. M. Hallerman, N. Umiel, G. Hulata, G. A.E. Gall, and B. May. **2000.** Breeding new strains of tilapia: development of an artificial center of origin and linkage map based on AFLP and microsatellite loci. Aquaculture. 185:43-56.

Belfiore, N.M. and B. May. **2000.** Variable microsatellite loci in red swamp crayfish, *Procambarus clarkii*, and their characterization in other crayfish taxa. Mol. Ecol. 12:2231-2234.

Ernest, H.B., M.C.T. Penedo, B. May, M. Syvanen, and W.M. Boyce. **2000.** Molecular tracking of mountain lions in the Yosemite Valley region in California: genetic analysis usng microsatellites and fecal DNA. Mol. Ecol. 9: 433-441.

Ludwig, A., B. May, L. Debus, and I, Jenneckens. **2000.** Heteroplasmy in the mtDNA Control Region of Sturgeon (*Acipenser*, *Huso* and *Scaphirhynchus*) Genetics 156:1933-1947.

McQuown, E.C., B.L. Sloss, R.J. Sheehan, J. Rodzen, G. Tranah, and B. May. **2000.** Microsatellite analysis of genetic variation in sturgeon: new primer sequences for *Scaphirynchus* and *Acipenser*. Trans. Am. Fish. Soc. 129:1380-1388.

KEVIN S. WILLIAMSON

Northwest Fisheries Science Center,
NOAA Fisheries
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Seattle, WA 98112-2097

Phone (206) 302-2428
Email Kevin.Williamson@noaa.gov

EDUCATION

Ph.D., Ecology / Conservation Genetics, University of California, Davis. 2004.
Dissertation research: Ecological genetics of fall-run Chinook salmon.
Advisor: Dr. Bernie May.

Bachelor of Science, Molecular Biology, University of California, San Diego, 1993.

QUALIFICATION SUMMARY

- Over eight years of laboratory research experience with biotech product development companies. Five years experience in applying molecular genetic techniques toward wildlife conservation issues.
- Major strengths in molecular biology include DNA sub-cloning & sequencing, PCR, cDNA library construction, Representational Difference Analysis, Southern hybridization, & oligonucleotide synthesis.
- Protein chemistry experience includes, but not limited to HPLC, Western analysis, protein purification & characterization, Immobilized Metal Affinity Chromatography (IMAC), affinity, ion exchange, & size exclusion chromatography.
- Development of ELISA/RIAs.

RESEARCH EXPERIENCE

Northwest Fisheries Science Center, NOAA Fisheries 11/04 – Present
Post-Doctoral Research as Research Molecular Geneticist

- Evaluating reproductive fitness between hatchery & naturally spawning spring-run Chinook salmon on the Wenatchee River.

- Investigating the underlying molecular genetic mechanism responsible for apparent sex-reversal of fall-run Chinook salmon observed in California.

Genomic Variation Lab, University of CA, Davis
Doctoral Research 7/99 – 10/04

- Applied genetic approach to evaluate potential alteration of progeny sex ratios from controlled breeding experiments with normal and apparent sex-reversed male, fall-run Chinook.

- Developed and characterized a suite of 17 microsatellite loci to be used for the evaluation of population genetic structure in fall-run Chinook salmon in the San Joaquin River Basin.

- Wrote quarterly and annual project reports to state and federal funding agencies to keep project supervisors updated on status of research programs.

- Supervised technicians and undergraduate assistant on project goals and advised on troubleshooting technical difficulties in a conservation genetics lab.

- Supervised safety training and oversaw all aspects of lab safety documentation as Laboratory Safety Officer for entire research group.

Science Staffing, Inc., San Diego, CA **1999**
Assignment-
Chugai Biopharmaceuticals, Inc.
Sr. Research Technician, Molecular Biology **1/99-6/99**

- Subcloned the human gene for Malonyl CoA Decarboxylase (MCD) into the mammalian expression vector pcDNA3.1 so that the MCD protein could be used as an immunigen for the production of antibodies, and as a reagent in developing a screening assay for potential inhibitors of MCD function.

- Set up a computerized FPLC system so that purification of rat heart MCD protein could be performed.

- Performed 5'-RACE to isolate a full-length mRNA transcript of the human heart version of the MCD gene.

NovaDx, Inc., San Diego, CA
RESEARCH ASSOCIATE, R and D 1995-1998

- Used PCR to evaluate the time course expression of both "novel" gene fragments as well as genes known to be expressed by osteoclasts in an in vitro bone resorption model. Verified, via PCR, the differential expression of "novel" gene fragments between the Giant Cell and Monocyte cDNA libraries used in Representational Difference Analysis (RDA) to find the "novel" gene fragments, and between a Paget's and a normal bone cDNA library. Constructed and performed Southern hybridization screening of osteoclast and Monocyte cDNA libraries.

- Designed PCR primers for the "novel" gene fragments produced through RDA between the Giant Cell & Monocyte cDNA libraries so that the above experiments could be carried out.

- Successfully cloned, bacterially expressed, IMAC purified, and characterized a potential osteoclast specific protein so that it may be used as an immunigen as well as a reagent for developing a diagnostic assay.

- Subcloned IL-2 & IL-4 cDNA from pBR322 into the mammalian expression vector pcDNA3 so that these constructs could be used for DNA vaccination of mice.

- Supervised radioisotope usage as Radiation Safety Officer for entire research group.

Hybritech, Inc., San Diego, CA **1988-1995**
RESEARCH ASSISTANT, DNA Probes Lab (1993-1995)

- Created a solution for an understaffed lab by streamlining DNA synthesis and purification and prioritizing equipment use. Reduced turnaround time between receipt of oligonucleotide synthesis request to delivered product by 50 percent.

- Verified the use of Isoelectric Focusing (IEF) electrophoresis as a means to evaluate DNA-Alkaline Phosphatase conjugate reactions so that rational decisions could be made on how to increase conjugation efficiency.

- Optimized a rapid method of purifying polyA-tailed DNA-Alkaline Phosphatase conjugates.

- 5' & 3'-end radiolabeled DNA and performed DNA sequencing (BRCA1 gene).

Laboratory Assistant, Assay Feasibility Lab (1990-1993)

- Reduced the amount of time required to obtain purified monoclonal antibodies thereby facilitating antibody characterization and assay development.

- Instituted an efficient monoclonal antibody purification protocol while eliminating an environmentally harmful by-product.

Laboratory Assistant, Product Support Lab (1988-1989)

PUBLICATIONS

Putative mutation revealed as a possible cause of apparent sex-reversal of male Chinook salmon in the Central Valley. Williamson, Kevin S., Bernie May (*in submission*). Transactions of the American Fisheries Society.

Homogenization of Fall-Run Chinook salmon gene pools in the Central Valley, California, USA. Williamson, Kevin S., Bernie May. North American Journal of Fisheries Management (*in press*).

Incidence of phenotypic female Chinook salmon (*Oncorhynchus tshawytscha*) positive for the male Y-chromosome specific marker OtY1 in the Central Valley, California, U.S.A. Williamson, Kevin S., Bernie May (2002). Journal of Aquatic Animal Health 14(3): 176-183.

Characterization of microsatellite loci in Chinook salmon (*Oncorhynchus tshawytscha*) and cross-species amplification in other salmonids. Williamson, Kevin S., Jan F. Cordes, Bernie May (2001). Molecular Ecology Notes 1(2): 17-19.

REPORTS AND ITEMS OF LIMITED DISTRIBUTION

Williamson, Kevin and B. May. Contaminant-induced sex-reversal of Fall-Run Chinook salmon (*Oncorhynchus tshawytscha*) in the Central Valley. Second annual report to U.S. Fish and Wildlife Service. September 2004. 40 pp.

Williamson, Kevin and B. May. Incongruence between sexual genotype and phenotype in Spring-Run Chinook salmon collected from Deer Creek in 1999. Report to Native Anadromous Fish and Watershed Branch, CA Dept. of Fish and Game. August 2004. 9 pp.

Williamson, Kevin and B. May. Contaminant-induced sex-reversal of Fall-Run Chinook salmon (*Oncorhynchus tshawytscha*) in the Central Valley. Annual report to U.S. Fish and Wildlife Service. September 2003. 12 pp.

Williamson, Kevin and B. May. Central Valley Fall-run Chinook genetic baseline Phase three final report to California Department of Fish and Game. June 2003. 157 pp.

Williamson, Kevin and B. May. Phase two of the San Joaquin Fall-Run Chinook Salmon Genetic Baseline Project final report to California Department of Fish and Game. February 2002. 7 pp.

RESEARCH FUNDING

I wrote the following funded proposals and contracts, conducted the research, and was listed as the Principal Investigator (PI) except where noted.

Total funding during Ph.D. research for which I wrote applications and proposals while working on Ph.D. (Grant, contract, scholarship, and fellowship funding) = $293,483, of which $17,140 = non-salary research funding and $276,343 = funding including salary & benefits.

CALFED: Sex-reversal in Central Valley Chinook salmon: occurrence and population genetic consequences. Funded: $212,936. 2002-2005. (PI Dr. Bernie May).

California Dept. of Fish and Game for San Joaquin River Basin Fall-Run Chinook Genetic Baseline and Discrimination. $294,000. 1999-2002. (PI Dr. Bernie May)
I collaborated on this project by conducting all research, writing quarterly and yearly reports and manuscripts for publication.

Marin Rod and Gun Club – Graduate Research Scholarship. Sex-reversal of male Chinook salmon in the Central Valley. $1500. 2002.

Jastro-Shields Scholarship (UC Davis research) - Validation of the genetic marker OtY1 to evaluate population level consequences of breeding by sex-reversed male Chinook salmon (*Oncorhynchus tshawytscha*) in California's Central Valley. $3,000. 2002.

Academic Fellowships, Scholarships total $82,718 2002-4.

Environmental Protection Agency Science To Achieve Results (STAR) Fellowship, 2003-4: Sex-reversal of male Chinook salmon in the Central Valley. Funded: $60,990.

Ecotoxicology Lead Campus Program Fellowship, 2002-3, Univ. of CA: Sex-reversal of male Chinook salmon in the Central Valley. Funded: $16,628.

Hart, Cole, Goss Graduate Fellowship. $4500. Summer 2002.

Hart, Cole, Goss Graduate Fellowship. $600. Summer 2003.

Graduate Group in Ecology Travel Award. $300. Summer 2004.

PAPER/POSTER PRESENTATIONS:

3rd Biennial CALFED Bay-Delta Program Science Conference, Sacramento, CA, 10/04- oral presentation entitled 'Sex-reversal of Fall Chinook: altered sexual differentiation or mutation?'.

STAR Fellowship Conference, Washington, D.C., 10/04- a poster regarding breeding experiments used to validate potential biomarkers of endocrine disrupting chemical exposure in Chinook salmon and the potential impact on salmon restoration was selected to be presented on Capital Hill at a reception co-sponsored by National Council for Science and the Environment, the American Chemistry Society, and the American Association for the Advancement of Science. Title: 'Apparent Sex-reversal of male Chinook salmon in California'

Coast-wide Salmonid Genetics Meeting, Newport, OR, 06/04- presented a talk entitled 'Sex-reversal of Fall Chinook: altered sexual differentiation or mutation?' at an international meeting focused specifically on salmonid genetics.

RUTH PHILLIPS: CURRICULUM VITAE JANUARY 2005

I. PERSONAL DATA:

Home Address: 14405 NE 26th Ave, Vancouver, WA 98686
Office Address: School of Biological Sciences, WSU, 14204 NE Salmon Creek Ave.
Vancouver, WA 98686-9600
Phone 360-546-9505/9504; Fax 360-546-9064; email: phllipsr@vancouver.wsu.edu

II. EDUCATION:

Swarthmore College, Swarthmore, Pennsylvania, B.A. with honors in Biology, 1962.
Indiana University, Bloomington, Indiana, M.A. in Zoology, 1964.
University of Illinois, Urbana, Illinois, Ph.D. in Genetics, 1967.
University of Illinois, Urbana, Illinois, Post doctoral trainee, 1967-68.

III. RESEARCH AND PROFESSIONAL EXPERIENCE:

Adjunct Research Professor, School of Biological Sciences, Washington State University, Vancouver, Washington, July 2001-present
Scientist, Center for Reproductive Biology, Washington State University, Pullman, Washington,July 2001-present
Affiliated Scientist, NIEHS Marine and Freshwater Biomedical Core Center, Oregon State University, January 2003-present
Professor emeritus, Department of Biological Sciences, University of Wisconsin-Milwaukee 2001-present (Professor, 1989-2001, Associate Professor 1975-1989, Assistant Professor 1971-1975, and Lecturer 1970-1971).
Visiting Professor, School of Fisheries, University of Washington, Seattle, Washington 95-96
Senior Scientist, NIEHS Marine and Freshwater Biomedical Core Center, University of Wisconsin-Milwaukee 1989-2001
Senior Scientist, Center for Great Lakes Studies, University of Wisconsin-Milwaukee, 1989-2001 (Associate scientist 1982- 1989)
Visiting Scientist, Fisheries Research Section, Ontario Ministry of Natural Resources, Maple, Ontario, September 1983 - December 1983.
Assistant Professor, Department of Zoology, University of Illinois, 1968-1970.

IV. FELLOWSHIPS AND AWARDS:

NSF Visiting Professorships for Women Award, 1995-96
UWM Foundation Research Award, 1991
NIH Postdoctoral Fellowship, 1967-68
NIH Predoctoral Fellowship, 1962-1966,
NSF Undergraduate Research Participation Fellowship 1960, 61

VI. PROFESSIONAL SOCIETIES:

American Association for the Advancement of Science, Genetics Society of America American Society of Human Genetics, American Society of Ichthyologists and

Herpetologists,American Fisheries Society, Society for the Study of Evolution Society for Molecular Biology and Evolution , Sigma Xi

VII. PUBLICATIONS (last ten years):

Book Chapters:

Phillips, R. B. 2005. Chromosome Morphology. *In* Stock Identification Methods, Applications in Fisheries Science, edited by Steve Cadrin, Kevin Friedland, and John Waldman. Elsevier Press, New York.

Phillips, R. B. and K. M. Reed. 2003. Chromosome Variation. *In* Genetic Principles and Practices for Fisheries Scientists. American Fisheries Society.

Phillips, R. B. and T. H. Oakley. 1997. Evolutionary relationships among salmonid fishes inferred from nuclear and mitochondrial sequences. Chapter 10. p145-162. *In* Molecular Systematics of Fishes, edited by Thomas Kocher and Carol Stepien, Academic Press.

Phillips, R.B., and P.E. Ihssen. 1990. Genetic marking of fish using variation in chromosomes and nuclear DNA. In N.C.Parker, A.E. Giogri, R.C. Heidinger, D.B. Jerter Jr., E.D. Prince, G.A.Winans (eds), Fish Marking Techniques. American Fisheries Symposium 7:499-513.

Phillips, R.B., L.E. Van Ert, K.A. Pleyte, and S.E. Hartley. 1989. Evolution of nucleolar organizer regions (NORs) and ribosomal RNA genes in fishes of the genus *Salvelinus*. In Kawanabe, H., Yamazaki, F., and D.L.G. Noakes (eds), Physiology and Ecology Japan. Biology of charrs and masu salmon.Special Volume 1:429-447.

2. Journal Articles:

Phillips, R. B. 2004. Adaptive evolution or genetic drift? Does genome complexity produce organismal complexity? (Invited commentary paper). Heredity 93:122-123.

Ohta, Y., Landis, E., Boulay, T., Phillips, R.B., Collet, B., Secombes, C.J., Flajnik, M. F., and Hansen, J.D. 2004. Homologs of CD83 from elasmobranch and teleost fish. J. Immunology 173:4553-4560.

Phillips, R. B., Noakes, M.A., Morasch, M., Felip, A. and G. H. Thorgaard. 2004. Does differential selection on the 5S rDNA explain why the rainbow trout sex chromosome heteromorphism is NOT linked to the SEX locus? Cytogenetics and Genome Research 105:122-125.

Felip, A., Fujiwara, A., Young, W.P., Wheeler, P. A., Noakes, M., Phillips, R.B. and G.H. Thorgaard. 2004. Polymorphism and differentiation of rainbow trout Y chromosomes (Genome, in press).

Phillips, R. B., Zimmerman, A., Noakes, M., Palti,Y. Morasch, M., Eiben, L., Ristow, S. , Thorgaard, G. H. and J. D. Hansen. 2003. Physical and genetic mapping of the rainbow trout major histocompatibility regions: evidence for duplication of the class I region. Immunogenetics 55:561-569.

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January 5, 2005

Ms. Kate Marie, Grant Manager
Science Program
California Bay-Delta Authority
650 Capitol Mall, 5th Floor
Sacramento CA 95814

Dear Ms. Marie:

Letter in Support of Project Entitled
"Are Apparent Sex Reversed *Chinook* Salmon a Symptom of Genotoxicity?"
Principal Investigator- Dr. Bernie P. May, UCD

It is our pleasure to forward institutional support and approval of the collaboration by UCD's Dr. May on the referenced research project to the California Bay-Delta Authority Science Program.

Please note as outlined in Attachments 3 and 6 of the Solicitation we would like to address the Compliance with Standard Terms and Conditions section in order to provide notification that UCD takes exception to the following proposed standard clauses:

Exhibit C – Section 12 – State Travel & Per Diem Expenses Guidelines (Delete)
Exhibit C – General Terms and Conditions for CBDA Grants (Replace with GIA 101)
Exhibit D – Special Terms and Conditions for CBDA Grants (Replace with UC IP Clause)

Please note the above has previously been negotiated with CALFED/GCAPS on behalf of the University of California and agreeable language has been included in current agreements with UC Davis.

Should the Department make an award to the University, we would anticipate negotiating terms that comply with University and federal guidelines as they pertain to the higher learning institutions and retention of intellectual property rights.

Please contact the principal investigator for scientific information. Administrative questions may be directed to me by telephone, facsimile or electronic mail at the numbers cited above.

Sincerely,

David Ricci
Contracts & Grants Analyst

cc: Dr. May

ARE 'APPARENT' SEX REVERSED CHINOOK SALMON A SYMPTOM OF GENOTOXICITY?: signature

This proposal is for the Science Program 2004 solicitation as prepared by May, Bernie P.

2004-12-27: In response to user feedback, the project and conflict of interest forms have been corrected. Please read the current versions carefully.

The applicant for this proposal must submit this form by printing it, signing below, and faxing it to +1 877-408-9310.

Failure to sign and submit this form will result in the application not being considered for funding.

The individual signing below declares that:

- all representations in this proposal are truthful;
- the individual signing the form is authorized to submit the application on behalf of the applicant (if applicant is an entity or organization);
- the applicant has read and understood the conflict of interest and confidentiality discussion under the Confidentiality and Conflict of Interest Section in the main body of the PSP and waives any and all rights to privacy and confidentiality1 of the proposal on behalf of the applicant, to the extent provided in this PSP; and
- the applicant has read and understood all attachments of this PSP.

proposal title: ARE 'APPARENT' SEX REVERSED CHINOOK SALMON A SYMPTOM OF GENOTOXICITY?

proposal number: 2004.01-0318

submitter: May, Bernie P (bpmay@ucdavis.edu)

applicant signature	date
	January 4, 2005
David Ricci Contracts and Grants Analyst	University of California, Davis
printed name of applicant	**applicant organization**

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